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DESCRIPTION

BLOOD BAG SYSTEM AND METHOD OF INACTIVATING PATHOGENIC MICROORGANISMS

TECHNICAL FIELD

The present invention relates to a method of inactivating pathogenic microorganisms in blood and to a blood bag system for supplying microbiologically and toxicologically safe blood preparations.

BACKGROUND ART

Blood transfusion is indispensable in modern medicine. Blood transfusion had caused 9,000 people to be infected with HIV until the American National Red Cross commenced screening to remove blood contaminated with HIV (human immunodeficiency virus) in 1985. Thereafter, blood preparations were screened for virus infection and infected blood preparations were discarded. As a result, the number of infected patients by transfusion was reduced to mere 41. Even so, however, it is impossible by the present screening technology to detect pathogenic microorganisms that cause infectious diseases by transfusion such as viruses and bacteria out of inspection items, and thus, the danger of

being infected with new virus or the like cannot be excluded. Accordingly, treatment for inactivating pathogenic microorganisms becomes necessary for blood preparations.

Known inactivation technologies for pathogenic microorganisms such as viruses and bacteria thus far developed include Solvent/Detergent (SD) treatment for blood preparations. The treatment has a disadvantage in that it is ineffective to nonenveloped viruses, and safe blood preparations are not necessarily supplied. For this reason, in recent years, inactivators against pathogenic microorganisms are being energetically developed in order to supply safe blood.

For example, virus inactivators containing an ethyleneimine oligomer has been proposed (International Publication Nos. WO97/07674 and WO99/17802). The ethylene oligomer is excellent for inactivation of viruses but has an unstable structure so that it evaporates at ambient temperature and explodes. Accordingly, use of the ethyleneimine oligomer for the inactivation of pathogenic microorganisms in blood preparations involves a high risk of infection of the operator.

Also, production and storage of the ethyleneimine oligomer requires maintenance at low temperatures, so that

the inactivators for pathogenic microorganisms containing an ethyleneimine oligomer not only require much cost for the production facility as well as transportation and storage, but also require further attention for safety management of the inactivators against pathogenic microorganisms containing an ethyleneimine oligomer when used at medical sites.

Therefore, it is an object of the present invention to provide a method of inactivating pathogenic microorganisms in blood free of various problems caused by the conventional inactivators against pathogenic microorganisms and a blood bag system for blood preparations in order to supply microbiologically and toxicologically safe blood preparations. In particular, an object of the present invention is to provide a blood bag system for blood preparations in which pathogenic microorganisms in blood are efficaciously inactivated and the inactivation effect is sustained over a long period of time and which is stable under environmental conditions such as light and temperature.

DISCLOSURE OF THE INVENTION

The above objects may be attained by the present invention described below.

The present invention provides a blood bag system including one or more of containers capable of holding a liquid, and a connecting tube connected liquid-tightly to the container, characterized in that: at least one of the containers holds an inactivator that inactivates a pathogenic microorganism contained in blood and/or an anticoagulant; and the inactivator contains as a main component a platinum compound capable of binding to nucleic acids of the microorganism or an aquo complex of the platinum compound.

According to the blood bag system of the present invention, two or more of the containers are provided, and the inactivator and the anticoagulant are held in different containers, respectively. The container holding the inactivator and the container holding the anticoagulant may be connected by the connecting tube.

Further, according to the blood bag system of the present invention, the inactivator and the anticoagulant may be held in the same container.

Therefore, the blood bag system of the present invention is a blood bag system including a blood bag holding an anticoagulant and a container holding an inactivator that inactivates a microorganism contained in blood, connected to each other, in which the inactivator

contains as a main component a platinum compound capable of binding to nucleic acid of the microorganism or an aquo complex of the platinum compound; or a blood bag system holding an anticoagulant and an inactivator that inactivates a microorganism contained in blood, in which the inactivator contains as a main component a platinum compound capable of binding to nucleic acid of the microorganism or an aquo complex of the platinum compound.

According to the blood bag system of the present invention, the platinum compound is preferably at least one selected from the group consisting of cisplatin, carboplatin, and nedaplatin.

According to the blood bag system of the present invention, the aquo complex of the platinum compound is at least one selected from the group consisting of a monoaquo complex (for example, cis-monochloromonoaqua diammineplatinous(II)chloride), a diaquo complex (for example, cis-diaquodiammineplatinous(II)dinitrate), a monoaquomonohydroxo complex (for example, cis-monohydroxymonoaqua diammineplatinous(II)chloride), and a dihydroxo complex (for example, cis-dihydroxydiammineplatinum(II)).

According to the blood bag system of the present invention, the pathogenic microorganism is preferably at

least one selected from the group consisting of DNA type viruses, RNA type enveloped viruses, and bacteria.

The present invention also provides a method of inactivating a pathogenic microorganism in blood, including: adding a microorganism inactivator containing as a main component a platinum compound capable of binding to nucleic acids of the microorganism or an aquo complex of the platinum compound to a blood bag that holds blood collected in advance.

According to the method of inactivating a pathogenic microorganism of the present invention, it is preferred that the microorganism inactivator is added so that a concentration becomes 0.07 mM (μmol/mL) to inactivate 1 $\log_{10}$ or more of the pathogenic microorganism in the blood held in the blood bag.

The method of inactivating a pathogenic microorganism of the present invention further preferably includes: adding a neutralizing agent containing as a main component an amino acid compound or a thiosulfate to neutralize the inactivator, after adding the microorganism inactivator.

According to the method of inactivating a pathogenic microorganism of the present invention, the neutralizing agent is preferably methionine or sodium thiosulfate.

According to the method of inactivating a pathogenic

microorganism of the present invention, the neutralizing agent is preferably added so that a concentration becomes 10 to 500 times a concentration of the microorganism inactivator.

Further, the present invention provides an inactivator that inactivates a microorganism contained in blood that is collected in advance containing as an active component a platinum compound or an aquo complex of the platinum compound.

According to the inactivator of the present invention, the platinum compound is preferably at least one selected from the group consisting of cisplatin, carboplatin, and nedaplatin.

Further, the present invention provides a method of using a platinum compound or an aquo complex of the platinum compound as an inactivator for a microorganism in blood that is collected in advance.

BRIEF DESCRIPTION OF DRAWINGS

Fig. 1 is a schematic diagram illustrating a blood bag system according to one construction example of the present invention;

Fig. 2 is a schematic diagram illustrating a blood bag system according to another construction example of the

present invention;

Fig. 3 is a graph illustrating SV-40 killing effect of a platinum compound in Example 1, with the SV-40 killing effect being shown as inactivation effect (logarithm ($\log_{10}$));

Fig. 4 is a graph illustrating SV-40 killing effect of a platinum compound in Example 2, with the SV-40 killing effect being shown as inactivation effect (logarithm ($\log_{10}$));

Fig. 5 is a graph illustrating an effect of cisplatin to inactivate *Yersinia enterocolitica*;

Fig. 6 is a graph illustrating an effect of neutralizing cisplatin in Example 6 with methionine; and

Fig. 7 is a graph illustrating an effect of neutralizing cisplatin in Example 6 with sodium thio sulfate.

BEST MODE FOR CARRYING OUT THE INVENTION

Hereinafter, the present invention will be described in detail.

A blood bag system of the present invention includes at least one container capable of holding a liquid, and a connecting tube liquid-tightly connected to the container capable of holding the liquid for use in charging and

discharging the liquid held in the container. At least one of the containers constituting the system holds an anticoagulant in order to hold collected blood. Hereinafter, the container holding the anticoagulant is referred to as a "blood bag". The blood bag system may have, in addition to the blood bag, one or more other containers for use in holding drugs and blood components such as erythrocytes, leukocytes, platelets, or plasma after separation.

Where the blood bag system includes a plurality of containers, the containers are connected to each other with connecting tubes, through which the liquid held in the containers, for example, blood is charged or discharged. Further, the blood bag system may include a connecting tube that is connected to the container at only one end of the connecting tube. Such a tube that is connected to the container at only one end thereof is used mainly for charging and discharging blood between the inside and the outside of the blood bag system. Specific example of such a tube includes a blood collection tube equipped with a needle at an end opposite to the end at which the container is connected.

Fig. 1 is a schematic diagram illustrating a blood bag system according to one construction example of the

present invention. In the blood bag system illustrated in Fig. 1, a blood bag 1, a container 2 holding an inactivator that inactivates a microorganism contained in blood, and a plurality of (two in Fig. 1) blood component bags 3 and 4 as the other containers are connected through connecting tubes 5. To the blood bag 1 is connected a blood collecting tube 6 equipped with a blood collecting needle 7 at the tip of the tube. The connecting tubes 5 can be sealed by pressing with an aluminum ring or by heat fusion using a heat sealer. Further, the blood component bags 3 and 4 for holding blood components after separation are designed so as to be readily detached separately.

Fig. 2 is a schematic diagram illustrating a blood bag system according to another construction example of the present invention. In the blood bag system shown in Fig. 2, the blood bag 1 and the container 2 holding an inactivator that inactivates a microorganism contained in blood are connected through the connecting tube 5. To prevent migration of the contents between the blood bag 1 and the container 2 unless necessary, the connecting tube 5 is provide with a valve 51 as an opening and closing means for the connecting tube 5. Note that though not shown, in the blood bag system illustrated in Fig. 1, it is preferable that the connecting tubes 5 that connect the

blood bag 1, the container 2, and the blood component bags 3 and 4 are provided with a klemme, which is an opening and closing means.

To the blood bag 1 is connected the blood collecting tube 6 equipped with the blood collecting needle 7 at the tip of the tube. Further, the blood bag 1 is provided with an air-bleeding tube 10. When the collected blood from the blood collecting tube 6 or the inactivator from the container 2 is introduced into the blood bag 1, air-bleeding of the air in the inside the blood bag 1 through a tube 10 facilitates the introduction of the blood or inactivator into the blood bag 1.

Each constituent element of the blood bag systems shown in Figs. 1 and 2 are preferably moldings of a thermoplastic resin such as a vinyl chloride resin containing a plasticizer, taking safety into consideration.

In the illustrated blood bag system, the blood bag 1 holds an anticoagulant, so that the coagulation of the collected blood can be prevented. The anticoagulant held in the blood bag 1 may be selected from a wide variety of known anticoagulants. Specific examples of the anticoagulant that can be used include citric acid, heparin, low molecular weight heparin, nafamostat mesilate, gabexate mesilate, and EDTA. The anticoagulants are

usually diluted with physiological saline or an aqueous glucose solution and is held in such an amount that the ratio of the anticoagulant liquid to the blood to be held is an ordinary ratio, specifically an amount of, for example, about (1:2 to 1:20). The anticoagulants may constitute a part of a blood preserving liquid. Examples of such a blood preserving liquid include physiological solution such as ACD liquid and CPD liquid that contains one or more of adenine, mannitol, sorbitol, guanosine, and the like.

The container 2 holds an inactivator that inactivates pathogenic microorganisms contained in blood. The inactivator as necessary is introduced into the blood bag 1 through the connecting tube 5 by opening preferably the valve 51, which is an opening and closing means. This inactivates the pathogenic microorganisms contained in the blood in the blood bag 1.

Note that the illustrated blood bag system assumes a construction in which the anticoagulant is held in the blood bag 1 and the inactivator is held in the container 2, that is, a construction in which the anticoagulant and the inactivator are held in different containers. However, the anticoagulant and the inactivator may be held in the same container. That is, in the illustrated construction, the

inactivator may be held in advance in the blood bag 1. In this case, inactivation of the pathogenic microorganisms contained in blood is performed as soon as the collected blood is introduced in to the blood bag 1.

However, in the case where a blood preserving liquid containing adenine and guanosine is used, mixing a platinum compound with adenine and guanosine before inactivation of the pathogenic microorganisms must be avoided since adenine and guanosine react with the platinum compound.

According to the present invention, the pathogenic microorganisms contained in blood and that need to be inactivated include DNA-type viruses, RNA enveloped viruses, and bacteria. Viruses are genetically classified into DNA type and RNA type. Structurally, the viruses are classified based on whether or not a viral membrane (envelope) is present. HIV and HCV (human hepatitis C virus) currently causing transfusion infection problems are RNA type enveloped viruses. HBV (human hepatitis B virus) is a DNA type enveloped virus.

Further, bacteria causing transfusion infection problems include *Yersinia enterocolitica* and *Serratia marcescens*. Blood microorganism level of patients infected with these bacteria is 1×10^1 to 1×10^2 CFU/mL. Blood collection using a blood bag containing no inactivator

results in an increase to 1×10^6 to 1×10^8 CFU/mL in about three weeks. Transfusion of blood having such a microorganism level may sometimes lead to death due to an endotoxin shock.

The present invention is characterized by using a platinum compound known as an anticancer agent, more specifically a platinum compound capable of binding to the nucleic acids of pathogenic microorganisms and/or an aquo complex of the platinum compound (both may be sometimes referred to simply as platinum compound) as a drug that inactivates the pathogenic microorganisms in blood for transfusion (hereinafter, referred to as "inactivator"). When the platinum compound is used as an inactivator, binding of the platinum compound to the nucleic acids of the pathogenic microorganisms is presumed to inactivate the pathogenic microorganisms.

The platinum compound used in the present invention may bind to the nucleic acids of the pathogenic microorganisms. Specific examples thereof include cisplatin, carboplatin, and nedaplatin. Two or more of these may be used in combination.

Further, in the present invention, aquo complexes of the platinum compounds, that is, aquo complexes of cisplatin, carboplatin, nedaplatin, or the like may be

used. Preferable aquo complexes include a monoaquo complex such as cis-monochloromonoaqua(II) chloride, a diaquo complex such as cis-diaquodiammineplatinous(II) dinitrate, a monoaquomonohydroxo complex such as cis-monohydroxymonoaqua diammineplatinous(II) chloride, and a dihydroxo complex such as cis-dihydroxydiammineplatinum(II). Two or more of these may be used in combination. Further, these may be used in combination with the above-mentioned platinum compounds.

It is preferable that the platinum compound is dissolved in physiological saline or an aqueous glucose solution and held in the container 2 in a liquid state having an osmotic pressure ratio of about 1 so that it can be introduced from the container 2 to the blood bag 1 through the connecting tube 5, preferably by opening the valve 51 as an opening and closing means.

Further, since the platinum compound is decomposed with light or its stability is changed depending on ion concentration, it is preferable that a light-shielded container is used as the container 2 holding the inactivator or that the ion concentration of the inactivator is adjusted with sodium chloride or the like before the inactivator is held in the container 2.

The method of inactivating pathogenic microorganisms

is to inactivate pathogenic microorganisms contained in blood by addition of an inactivator containing as a main component the above-mentioned platinum compound or an aquo complex of the platinum compound to the blood bag holding blood in advance. The method is preferably practiced by using the blood bag system of the present invention as mentioned above. Hereinafter, the method of inactivating pathogenic microorganisms according to the present invention will be described by using the blood bag system illustrated in Fig. 2.

Specifically, in the blood bag system of Fig. 2, the inactivator is introduced from the container 2 into the blood bag 1 that holds blood collected through the blood collecting needle 7 and the blood collecting tube 6 by opening the valve 51 attached to the connecting tube 5, thus practicing the method of inactivating pathogenic microorganisms according to the present invention.

According to the method of the present invention, the amount of platinum compound to be used may vary depending on time, temperature, composition and concentration of blood components and so on. At the time of inactivation, the pathogenic microorganisms in blood held in the blood bag can be inactivated even when a final concentration of the platinum compound in the blood bag 1 is about 0.02 mM

(μmol/mL), specifically 0.0209 mM. However, the final concentration of the platinum compound in the blood bag 1 is preferably 0.07 to 100 mM, more preferably 1 to 10 mM. If the final concentration of the platinum compound within the above-mentioned concentration range, the effect of inactivating the pathogenic microorganisms in blood is high and specifically the pathogenic microorganisms in blood can be inactivated by 1 $\log_{10}$ or more. If the final concentration of the platinum compound is above 100 mM, the platinum compound cannot be readily dissolved depending on the solvent so that it is difficult to prepare a solution.

Note that the term "final concentration of platinum compound" as used herein refers to a concentration (mM) of a platinum compound at the time when substantially all of the platinum compound to be used is introduced into the blood bag 1 upon the inactivation treatment.

Specifically showing the amount of the platinum compound to be used in the method of the present invention, when the platinum compound is cisplatin and the pathogenic microorganisms in a concentrated erythrocyte preparation (RC-MAP) are to be inactivated, the platinum compound is used in an amount so that a final concentration becomes 21 μg/mL to 30 mg/mL, preferably 0.3 mg/mL to 3 mg/mL.

Note that the effective concentration of cisplatin

against cancer cells is 0.25 μg/mL (antiviral activity (IC_{50}) on initial culture cells of Ehrlich cancer), and the above-mentioned final concentration of cisplatin of 21 μg/mL is as high as 84 times. That is, in the case of blood preparations, cisplatin is used in concentrations much higher than the concentration in which it is used as an anticancer agent to humans.

According to the method of the present invention, the temperature at which the platinum compound is added to blood for a reaction between the platinum compound and the nucleic acids of pathogenic microorganisms only needs to be 0°C or higher but is preferably 4 to 30°C in consideration of influences on blood preparations.

Also, according to the present invention, upon addition of the platinum compound to blood, the reaction between the platinum compound and the nucleic acids of pathogenic microorganisms starts at once. The reaction is preferably continued for 3 hours or more in order to effect the inactivation of the pathogenic microorganisms more completely.

Since the platinum compound used in the method of the present invention belongs to poison in toxicology and since the platinum compound are used in very high concentration as mentioned above, neutralization treatment is preferably

performed after the inactivation treatment in order to alleviate the toxicity of the platinum compound. In such neutralization treatment, a neutralizing agent that is usually used in converting the toxicity of anticancer agents containing the platinum compounds to toxicologically safe is preferably added to blood preparations containing the platinum compound after inactivation. Also, the neutralization treatment may be performed by irradiation of light. Note that the neutralization treatment also involves decreasing the reactivity of the platinum compound with the nucleic acids of the pathogenic microorganisms, and thus, can serve to terminate the inactivation reaction.

To add the neutralizing agent to blood preparations containing the platinum compound after inactivation reaction for a desired period of time, for example, a container containing a neutralizing agent is connected in advance to the blood bag 1 through a connecting tube having a valve as an opening and closing means and the valve is opened as necessary to introduce the neutralizing agent into the blood bag 1. Also, a neutralizing agent may be introduced into the blood bag 1 from the air-bleeding tube 10 by using a syringe or the like.

A reaction of the neutralizing agent with the platinum compound, as a microorganism inactivator, in a

concentration 42 times or higher the concentration of the platinum compound can result in complete neutralization treatment. Usually, the neutralizing agent is added in a concentration 10 to 500 times, preferably 25 to 100 times the concentration of the platinum compound.

Although the neutralizing treatment is effective at a temperature of 0°C or higher, the reaction is preferably performed at a temperature of 4 to 30°C in consideration of influences on the function of blood preparations.

Further, although the reaction starts immediately after addition of the platinum compound, the reaction is preferably continued for about 30 minutes to about 1 hour in order to ensure the neutralization treatment.

Examples of the neutralizing agent for cisplatin include thiosulfates such as sodium thiosulfate, amino acids such as methionine and cysteine, and thiol compounds such as glutathione and coenzyme A, with sodium thiosulfate and methionine being preferable. To conveniently introduce the neutralizing agent into the blood bag 1, the neutralizing agent is preferably liquid added in a state having an osmotic pressure ratio of about 1 after dissolving the neutralizing agent in physiological saline or an aqueous glucose solution.

Note that photodegradable neutralizing agent such as

methionine is stored preferably in a light-shielding container.

The method of inactivating pathogenic microorganisms according to the present invention by using the blood bag systems illustrated in Figs. 1 and 2 is described above, the method of the present invention is not limited thereto. For example, in the case where the blood bag system of the present invention holds an anticoagulant and an inactivator in the blood bag in advance, the inactivation reaction of the pathogenic microorganisms in blood starts at the time when the collected blood is introduced into the blood bag. Further, the method of the present invention may be performed by introducing the inactivator into the blood bag that holds blood in advance by using a syringe or the like.

Therefore, the present invention also includes the inactivator for pathogenic microorganisms in blood collected in advance containing as active components the above-mentioned platinum compound and aquo complex of the platinum compound, and a method of using the above-mentioned platinum compound and aquo complex of the platinum compound as inactivators for pathogenic microorganisms in blood collected in advance.

EXAMPLES

Hereinafter, the present invention will be described in detail by examples. However, the present invention should not be considered as being limited thereto.

(Example 1)

[Virus inactivation for simian virus 40 (SV-40) which is a DNA type nonenveloped virus]

In a 400-mL CPDA blood bag in the blood bag system shown in Fig. 1, RC-MAP (concentrated erythrocyte preparation) was prepared from blood collected from healthy humans. Thereafter, 2 mL of RC-MAP was sampled in a 15-mL centrifuging tube aseptically, and 2 mL of 10^8 $TCID_{50}$/mL (50% infection end point) SV-40 was added thereto, followed by gentle stirring. To the resultant was added 2 mL of a cisplatin solution containing 1.67 mM cisplatin in an aqueous solution containing sodium chloride and hydrochloric acid (Briplatin (registered trademark)) as an inactivator, followed by gently stirring and a reaction at room temperature (in the dark) for 7 hours to effect inactivation. After completion of the inactivation, the inactivated solution was centrifuged at 3,000 rpm for 1 minute and the supernatant was used for the evaluation of virus inactivation.

Further, inactivation was similarly performed using, instead of the cisplatin solution, a 3.5 mM carboplatin

solution having dissolved carboplatin (Paraplatin (registered trademark)) in a phosphate buffer and a 3.5 mM nedaplatin solution having dissolved nedaplatin (Aqupla (registered trademark) in a phosphate buffer as inactivators and the supernatants of centrifuged inactivated solutions were used for the evaluation of virus inactivation.

Evaluation of virus inactivation was performed by $TCID_{50}$ using a 96-well microplate. In a 25-cm^2 cell culture flask, a CV-1 cell (simian kidney derived cell), which is a proliferating cell of SV-40, was cultivated in a 10% fetal bovine serum-added MEM medium for 3 days for proliferation. Thereafter, the cells were floated with 0.5 mL of 0.05% trypsin-EDTA, to prepare a cell suspension in 25 mL of the 10% fetal bovine serum-added MEM medium.

After 90 μL of the cell suspension was added to each well of the 96-well microplate, 10 μL of the reaction mixture (the above-mentioned supernatant) was added to each well of the first column of the 96-well microplate and pipetting was performed to stir the resultant. Further, 10 μL of the solution in each well of the first column was added to each well of the second column of the microplate to perform 10-fold gradual dilution operation. This operation was repeated up to the wells of the twelfth

column of the microplate. The microplate was incubated in a 5% carbon dioxide gas incubator at 37°C for 10 days. Then, a cytopathic effect (CPE) by SV-40 was observed using an optical microscope, and $TCID_{50}$ were calculated as logarithmic values (log_{10}).

At the same time, $TCID_{50}$ of a positive control, in which a phosphate buffer was added instead of the platinum compound, was calculated as a logarithmic value (log_{10}). A value obtained by subtracting $TCID_{50}$ of the inactivated solution from $TCID_{50}$ of the positive control was defined as a killing effect.

The results are shown in Fig. 3. In Fig. 3, the inactivation effect means a value obtained by subtracting $TCID_{50}$ of the inactivated solution (logarithmic value (log_{10})) from $TCID_{50}$ of the above-mentioned positive control (logarithmic value (log_{10})).

Here, $TCID_{50}$ of the positive control was 6.5 log_{10} and $TCID_{50}$ of the inactivated solutions obtained by reacting with the cisplatin solution, carboplatin solution, and nedaplatin solution, respectively, as the inactivators were 1.25 log_{10}, 5.50 log_{10}, and 2.25 log_{10}, respectively. The results obtained by subtracting the latter from the former confirmed that the 1.67 mM cisplatin solution had an inactivation effect of 5.25 log_{10}, the 3.5 mM carboplatin

solution had an inactivation effect of 1.00 $\log_{10}$, and the 3.5 mM nedaplatin solution had an inactivation effect of 4.25 $\log_{10}$.

(Example 2)

[Virus inactivation for simian virus 40 (SV-40) which is a DNA type nonenveloped virus using a blood bag system of the present invention]

Blood was collected from healthy humans and RC-MAP (concentrated erythrocyte preparation) was prepared, which was introduced into the blood bag 1 (400-mL CPDA blood bag) in the blood bag system shown in Fig. 2. Thereafter, 10^8 $TCID_{50}$/mL (50% infection endpoint) of SV-40 per 2 mL of RC-MAP was added to the blood bag 1 from the air-bleeding tube 10 by using a syringe, and the blood bag 1 was gently stirred.

Then, by opening the valve 51, a cisplatin solution containing 1.67 mM (μmol/mL) cisplatin in an aqueous solution containing sodium chloride and saline (Briplatin (registered trademark), available from Bristol Myers Squibb company) was introduced into the blood bag 1 in an amount of 2 mL per 2 mL of RC-MAP. Thereafter, the blood bag was stirred gently, and the resultant was allowed to react at room temperature (in the dark) for 7 hours to effect inactivation. After completion of the inactivation, 4 mL

of the inactivated solution was sampled from the blood bag 1 and centrifuged at 3,000 rpm for 1 minute. The supernatant was used for the evaluation of virus inactivation.

Further, inactivation was similarly performed using, instead of the cisplatin solution, a 3.5 mM carboplatin solution having dissolved carboplatin (Paraplatin (registered trademark), Bristol Pharmaceuticals K.K.) in a phosphate buffer and a 3.5 mM nedaplatin solution having dissolved nedaplatin (Aqupla (registered trademark), Shionogi & Co., Ltd.) in a phosphate buffer as inactivators and the supernatants of centrifuged inactivated sampled from the blood bag 1 were used for the evaluation of virus inactivation.

Evaluation of virus inactivation was performed by $TCID_{50}$ using a 96-well microplate. In a 25-cm^2 cell culture flask, a CV-1 cell (simian kidney derived cell), which is a proliferating cell of SV-40, was cultivated in a 10% fetal bovine serum-added MEM medium for 3 days for proliferation. Thereafter, the cells were floated with 0.5 mL of 0.05% trypsin-EDTA, to prepare a cell suspension in 25 mL of the 10% fetal bovine serum-added MEM medium.

After 90 μL of the cell suspension was added to each well of the 96-well microplate, 10 μL of the reaction

mixture (the above-mentioned supernatant) was added to each well of the first column of the 96-well microplate and pipetting was performed to stir the resultant. Further, 10 μL of the solution in each well of the first column was added to each well of the second column of the microplate to perform 10-fold gradual dilution operation. This operation was repeated up to the wells of the twelfth column of the microplate. The microplate was incubated in a 5% carbon dioxide gas incubator at 37°C for 10 days. Then, a cytopathic effect (CPE) by SV-40 was observed using an optical microscope, and $TCID_{50}$ were calculated as logarithmic values (log_{10}).

At the same time, $TCID_{50}$ of a positive control, in which a phosphate buffer was added instead of the platinum compound, was calculated as a logarithmic value (log_{10}). A value obtained by subtracting $TCID_{50}$ of the inactivated solution from $TCID_{50}$ of the positive control was defined as a killing effect (inactivation effect).

The results are shown in Fig. 4. In Fig. 4, the inactivation effect means a value obtained by subtracting $TCID_{50}$ of the inactivated solution (logarithmic value (log_{10})) from $TCID_{50}$ of the above-mentioned positive control (logarithmic value (log_{10})).

Here, $TCID_{50}$ of the positive control was 6.0 log_{10} and

$TCID_{50}$ of the inactivated solutions obtained by reacting with the cisplatin solution, carboplatin solution, and nedaplatin solution, respectively, as the inactivators were 1.0 $\log_{10}$, 4.5 $\log_{10}$, and 2.5 $\log_{10}$, respectively. As the results obtained by subtracting the latter from the former, Fig. 4 shows that the 1.67 mM cisplatin solution had an inactivation effect of 5.0 $\log_{10}$, the 3.5 mM carboplatin solution had an inactivation effect of 1.5 $\log_{10}$, and the 3.5 mM nedaplatin solution had an inactivation effect of 3.5 $\log_{10}$.

(Example 3)

[Virus inactivation for Human cytomegalovirus (HCMV) which is a DNA type enveloped virus]

In the blood bag 1 (a 400-mL CPDA blood bag) in the blood bag system shown in Fig. 1, RC-MAP (concentrated erythrocyte preparation) was prepared from blood collected from healthy humans. Thereafter, 2 mL of RC-MAP was sampled in a 15-mL centrifuging tube aseptically, and 2 mL of 10^7 $TCID_{50}$/mL HCMV was added thereto, followed by gentle stirring. To the resultant was added 2 mL of a cisplatin solution containing 1.67 mM cisplatin in an aqueous solution containing sodium chloride and hydrochloric acid (Briplatin (registered trademark)) as an inactivator, followed by gently stirring and a reaction at room

temperature (in the dark) for 7 hours to effect inactivation. After completion of the inactivation, the inactivated solution was centrifuged at 3,000 rpm for 1 minute and the supernatant was used for the evaluation of virus inactivation.

Evaluation of virus inactivation was performed by $TCID_{50}$ using a 96-well microplate. In a 25-cm^2 cell culture flask, a HEL cell (primary human embryonic lung fibroblasts cell), which is a proliferating cell of HCMV, was cultivated in a 10% fetal bovine serum-added MEM medium for 3 days for proliferation. Thereafter, the cells were floated with 0.5 mL of 0.05% trypsin-EDTA, to prepare a cell suspension in 25 mL of the 10% fetal bovine serum-added MEM medium.

After 90 µL of this cell suspension was added to each well of the 96-well microplate, 10 µL of the solution (the above-mentioned supernatant) was added to each well of the first column of the 96-well microplate and pipetting was performed to stir the resultant. Further, 10 µL of the solution in each well of the first column was added to each well of the second column of the microplate to perform 10-fold gradual dilution operation. This operation was repeated up to the wells of the twelfth column of the microplate. The microplate was incubated in a 5% carbon

dioxide gas incubator at 37°C for 14 days. Then, a cytopathic effect (CPE) by HCMV was observed using an optical microscope, and $TCID_{50}$ was calculated as a logarithmic value (log_{10}). At the same time, $TCID_{50}$ of a positive control, in which a phosphate buffer was added instead of the platinum compound, was calculated as a logarithmic value (log_{10}). A value obtained by subtracting $TCID_{50}$ of the inactivated solution from $TCID_{50}$ of the positive control was defined as an anti-HCMV effect of cisplatin (inactivation effect).

Here, $TCID_{50}$ of the positive control was 5.25 log_{10} and $TCID_{50}$ of the inactivated solution obtained by reacting with the cisplatin solution was 1.25 log_{10}. The results obtained by subtracting the latter from the former confirmed that the 1.67 mM cisplatin solution killed 4.00 log_{10} of HCMV in RC-MAP.

(Example 4)

[Virus inactivation for mouse hepatitis virus (MHV) which is an RNA type enveloped virus]

In the blood bag 1 (a 400-mL CPDA blood bag) in the blood bag system shown in Fig. 1, RC-MAP (concentrated erythrocyte preparation) was prepared from blood collected from healthy humans. Thereafter, 2 mL of RC-MAP was sampled in a 15-mL centrifuging tube aseptically, and 2 mL

of 10^7 $TCID_{50}$/mL MHV was added thereto, followed by gentle stirring. To the resultant was added 2 mL of a cisplatin solution containing 1.67 mM cisplatin in an aqueous solution containing sodium chloride and hydrochloric acid (Briplatin (registered trademark)) as an inactivator, followed by gently stirring and a reaction at room temperature (in the dark) for 7 hours to effect inactivation. After completion of the inactivation, the inactivated solution was centrifuged at 3,000 rpm for 1 minute and the supernatant was used for the evaluation of virus inactivation.

Evaluation of virus inactivation was performed by $TCID_{50}$ using a 96-well microplate. In a 25-cm^2 cell culture flask, a DBT cell, which is a proliferating cell of MHV, was cultivated in a 5% fetal bovine serum-added MEM medium for 3 days for proliferation. Thereafter, the cells were floated with 0.5 mL of 0.05% trypsin-EDTA, to prepare a cell suspension in 25 mL of the 10% fetal bovine serum-added MEM medium.

After 90 µL of this cell suspension was added to each well of the 96-well microplate, 10 µL of the solution was added to each well of the first column of the 96-well microplate and pipetting was performed to stir the resultant. Further, 10 µL of the solution in each well of

the first column was added to each well of the second column of the microplate to perform 10-fold gradual dilution operation. This operation was repeated up to the wells of the twelfth column of the microplate. The microplate was incubated in a 5% carbon dioxide gas incubator at 37°C for 14 days. Then, a cytopathic effect (CPE) by MHV was observed using an optical microscope, and $TCID_{50}$ was calculated as a logarithmic value (log_{10}). At the same time, $TCID_{50}$ of a positive control, in which a phosphate buffer was added instead of the platinum compound, was calculated as a logarithmic value (log_{10}). A value obtained by subtracting $TCID_{50}$ of the inactivated solution from $TCID_{50}$ of the positive control was defined as an MHV inactivation effect.

Here, $TCID_{50}$ of the positive control was 5.50 log_{10} and $TCID_{50}$ of the inactivated solution obtained by reacting with the cisplatin solution was 1.25 log_{10}. The result obtained by subtracting the latter from the former confirmed that the 1.67 mM cisplatin solution killed 4.00 log_{10} of MHV in RC-MAP.

(Example 5)

[Bacteria inactivation of *Yersinia enterocolitica* (*Yersinia* strain)]

In the blood bag 1 (400-mL CPDA blood bag) of the

blood bag system shown in Fig. 1, RC-MAP (concentrated erythrocyte preparation) was prepared from blood collected from healthy humans. Thereafter, 2 mL of RC-MAP was aseptically sampled in a 15-mL centrifuging tube, and 2 mL of 10^8 CFU/mL *Yersinia enterocolitica* was added thereto, followed by gently stirring. To the resultant was added 2 mL of a cisplatin solution having dissolved cisplatin (available from Wako Pure Chemical Industries, Ltd.) in physiological saline and adjusted to a concentration of 4.00 mM, as an inactivator. After gentle stirring, the resultant was allowed to react at room temperature (in the dark) for 7 hours to effect inactivation. After completion of the inactivation, the inactivated solution was centrifuged at 3,000 rpm for 1 minute and the supernatant was used for the evaluation of virus inactivation.

Similarly, cisplatin solutions having dissolved cisplatin (available from Wako Pure Chemical Industries, Ltd.) in physiological saline and adjusted to concentrations of 2.00 mM, 1.00 mM, 0.50 mM, 0.25 mM, 0.125 mM, and 0.0625 mM were used as inactivators to effect inactivation, and the supernatants were used for the evaluation of bacteria inactivation.

Evaluation of antibacterial activity was performed on 10-fold gradually diluted solutions obtained by adding 1 mL

of the inactivated solution to 9 mL of sterilized physiological saline, stirring the mixture by using a touch mixer, and further adding 1 mL of the resultant to 9 mL of physiological saline for dilution. 1 mL or 100 μL of the diluted solution in each stage was mixed in a petri dish having a diameter of 9 cm and containing 15 mL soybean casein digest agar medium. This was cultivated at 31°C for 2 days. The number of colonies emerged was defined as number of survival bacteria.

Note that solution to which no cisplatin was added was defined as a positive control. A bacteria inactivation effect was defined by a value obtained by subtracting the number of survival bacteria (logarithmic value ($\log_{10}$)) in cisplatin-added inactivated solution from the number of survival bacteria (logarithmic value ($\log_{10}$)) in the positive control.

The results are shown in Fig. 5. Here, the number of survival bacteria was 5.00 $\log_{10}$, and the numbers of survival bacteria in the inactivated solutions inactivated by the cisplatin solutions are as follows.

Concentration of Cisplatin	Number of survival bacteria
1.33 mM	0.06 $\log_{10}$

0.67 mM	1.48 $\log_{10}$
0.33 mM	2.27 $\log_{10}$
0.17 mM	2.80 $\log_{10}$
0.083 mM	3.79 $\log_{10}$
0.042 mM	4.52 $\log_{10}$
0.0209 mM	4.82 $\log_{10}$

As a result of subtracting the latter from the former, the bacteria killing effect of each inactivator was as follows.

Concentration of cisplatin in inactivator	Bacteria killing effect
1.33 mM	4.94 $\log_{10}$
0.67 mM	3.52 $\log_{10}$
0.33 mM	2.73 $\log_{10}$
0.17 mM	2.20 $\log_{10}$
0.083 mM	1.21 $\log_{10}$
0.042 mM	0.48 $\log_{10}$
0.0209 mM	0.18 $\log_{10}$

(Example 6)

[Detoxication of cisplatin/anti-*Yersinia enterocolitica* activity after reaction of cisplatin with sodium

thiosulfate or methionine]

In the blood bag 1 (400-mL CPDA blood bag) of the blood bag system shown in Fig. 1, RC-MAP (concentrated erythrocyte preparation) was prepared from blood collected from healthy humans. Thereafter, 2 mL of RC-MAP was aseptically sampled in a 15-mL centrifuging tube and 2mL of a cisplatin solution having dissolved cisplatin (available Wako Pure Chemical Industries, (Ltd.)) in physiological saline and adjusted to a concentration of 4.00 mM as an inactivation was added, followed by gentle stirring. To the resultant was added 2 mL of a 168 mM L-methionine solution having dissolved L-methionine in physiological saline, for a reaction at room temperature for 30 minutes.

Similarly, as neutralizing agents, L-methionine solutions having concentrations of 84 mM, 42 mM, 21 mM, 10.5 mM, 5.25 mM, 2.63 mM, and 1.31 mM, respectively, obtained by dissolving L-methionine in physiological saline or sodium thiosulfate solutions having concentrations of 168 mM, 84 mM, 42 mM, 21 mM, 10.5 mM, 5.25 mM, 2.63 mM, and 1.31 mM, respectively, obtained by dissolving sodium thiosulfate in physiological saline were used to effect a reaction. In addition, solutions to which a phosphate buffer was added instead of the inactivator and neutralizing agent (without cisplatin) were prepared as

controls, and the reaction was performed in the similar manner. Thereafter, 10 μL of 10^8 CFU/mL *Yersinia enterocolitica* was added to each solution, and the number of bacterial cells after stirring was measured.

Measurement of bacterial cells was conducted on solutions obtained by repeating 3 times the 10-fold gradual dilution operation consisting of adding 1 mL of the inactivated solution to 9 mL of sterilized physiological saline, stirring the mixture by using a touch mixer, and further adding 1 mL of the resultant to 9 mL of physiological saline for dilution. 10 mL, 1 mL or 100 μL of the diluted solution in each stage was mixed in a petri dish having a diameter of 9 cm and containing 15 mL soybean casein digest agar medium. This was cultivated at 31°C for 2 days. The number of colonies emerged was defined as number of survival bacteria, and a logarithmic value ($\log_{10}$) thereof was defined as a neutralization effect.

The results of neutralization treatment with methionine as a neutralizing agent are shown in Fig. 6. The results confirmed that in the case where a neutralizing agent having a methionine concentration of 56 mM showed the same result as that obtained without cisplatin (number of survival bacteria of 5.1 $\log_{10}$) and *Yersinia enterocolitica* were not killed at all. In other cases where the

neutralizing agent was used in different concentrations, the numbers of survival bacteria were as follows.

Concentration of methionine in neutralizing agent	Number of survival bacteria
28 mM	3.36 $\log_{10}$
14 mM	1.87 $\log_{10}$
7 mM	1.43 $\log_{10}$
3.5 mM	1.22 $\log_{10}$
1.75 mM	0.89 $\log_{10}$
0.88 mM	0.73 $\log_{10}$
0.44 mM	0.34 $\log_{10}$

On the other hand, the results of the neutralization treatment with sodium thiosulfate as a neutralizing agent are shown in Fig. 7. The results confirmed that in the case where a neutralizing agent having a sodium thiosulfate concentration of 56 mM showed the same result as that obtained without cisplatin (number of survival bacteria of 5.1 $\log_{10}$) and *Yersinia enterocolitica* were not killed at all. In other cases where the neutralizing agent was used in different concentrations, the numbers of survival bacteria were as follows.

Concentration of sodium thiosulfate in neutralizing agent	Number of survival bacteria
28 mM	4.22 $\log_{10}$
14 mM	2.35 $\log_{10}$
7 mM	1.98 $\log_{10}$
3.5 mM	1.51 $\log_{10}$
1.75 mM	1.28 $\log_{10}$
0.88 mM	0.86 $\log_{10}$
0.44 mM	0.44 $\log_{10}$

(Example 7)

[Neutralization treatment of cisplatin/anti-SV-40 activity after reaction of cisplatin with sodium thiosulfate or methionine]

In the blood bag 1 (400 mL CPDA blood bag) of the blood bag system shown in Fig. 1, RC-MAP (concentrated erythrocyte preparation) was prepared from blood collected from healthy humans. Thereafter, 1 mL of RC-MAP was sampled in a 15-mL centrifuging tube aseptically and 1 mL of a cisplatin solution having dissolved cisplatin (available from Wako Pure Chemical Industries, Ltd.) in physiological saline and adjusted to a concentration of 3.5

mM cisplatin was added thereto as an inactivator, followed by gentle stirring. To the resultant was added 1 mL of a 175 mM sodium thiosulfate solution having dissolved sodium thiosulfate in physiological saline, for a reaction at room temperature for 30 minutes. Thereafter, 0.5 mL of 10^8 $TCID_{50}$/mL SV-40 was added and the resultant was stirred gently and then allowed to react in an incubator (in the dark) at room temperature for 30 minutes. After completion of the reaction, the resultant mixture was centrifuged at 3,000 rpm for 1 minute and the supernatant was used for the evaluation of virus inactivation.

Evaluation of virus inactivation was performed by $TCID_{50}$ using a 96-well microplate. In a 25-cm^2 cell culture flask, a CV-1 cell (simian kidney derived cell), which is a proliferating cell of SV-40, was cultivated in a 10% fetal bovine serum-added MEM medium for 3 days for proliferation. Thereafter, the cells were floated with 0.5 mL of 0.05% trypsin-EDTA, to prepare a cell suspension in 25 mL of the 10% fetal bovine serum-added MEM medium.

After 90 μL of the cell suspension was added to each well of the 96-well microplate, 10 μL of the reaction mixture (the above-mentioned supernatant) was added to each well of the first column of the 96-well microplate and pipetting was performed to stir the resultant. Further, 10

μL of the solution in each well of the first column was added to each well of the second column of the microplate to perform 10-fold gradual dilution operation. This operation was repeated up to the wells of the twelfth column of the microplate. The microplate was incubated in a 5% carbon dioxide gas incubator at 37°C for 10 days. Then, a cytopathic effect (CPE) by SV-40 was observed using an optical microscope, and $TCID_{50}$ was calculated as a logarithmic value ($\log_{10}$). At the same time, $TCID_{50}$ of a positive control, in which a phosphate buffer was added instead of the platinum compound was calculated as a logarithmic value ($\log_{10}$). A value obtained by subtracting $TCID_{50}$ of the inactivated solution from $TCID_{50}$ of the positive control was defined as a killing effect.

As a result, subtraction of $TCID_{50}$ (6.00 $\log_{10}$) of the case where a cisplatin solution having a concentration of 3.5 mM as an inactivator and then a sodium thiosulfate solution having a concentration of 175 mM as a neutralizing agent were added from $TCID_{50}$ (6.00 $\log_{10}$) of the positive control indicates no killing effect, that is, inactivation effect of SV-40. This means that the inactivator containing cisplatin in a concentration of 3.5 mM was neutralized by addition of the neutralizing agent containing sodium thiosulfate in a concentration of 175 mM.

(Example 8)

[Hemolysis toxicity of cisplatin on erythrocyte preparation]

In the blood bag 1 (400-mL CPDA blood bag) of the blood bag system shown in Fig. 1, RC-MAP (concentrated erythrocyte preparation) was prepared from blood collected from healthy humans. Thereafter, 1 mL of RC-MAP was aseptically sampled in a 15-mL centrifuging tube, and 1 mL of a cisplatin solution having dissolved cisplatin (Briplatin (registered trademark)) in physiological saline in a concentration of 1.67 mM and 1 mL of physiological saline were added thereto while gently mixing. The resultant was allowed to react at room temperature (in the dark) for 7 hours. Thereafter, the resultant mixture was centrifuged at 2,000 rpm for 5 minutes. Drabkin's reagent was added to the supernatant for a reaction and absorbance at 540 nm was measured to determine hemolysis rate by a cyanmethemoglobin method. As a result, the hemolysis rate of cisplatin was found to be 0.1% or less, confirming that cisplatin has low hemolysis toxicity on erythrocyte preparations.

INDUSTRIAL APPLICABILITY

The present invention employs a platinum compound

that is used as an anticancer agent and whose characteristics are widely known as an inactivator for pathogenic microorganisms in blood, and thus, the present invention is safe and the inactivator is easily available. Further, since there is no need to newly develop inactivators, the present invention is excellent in view of cost.

Further, use of the platinum compound in the present invention in a concentration as high as 0.07 mM or more, which is much higher than the amount of the platinum compound used as original use of anticancer agent, increases an effect of inactivating pathogenic microorganisms and provides an excellent sustained inactivation effect.

In the present invention, transfusion of inactivated blood after neutralization treatment of the anticancer agent and washing enables supply of microbiologically and toxicologically safe blood preparations unlike the original usage of anticancer agents used for the treatment of cancers.

In the present invention, blood or blood components containing inactivated pathogenic microorganisms and preferably further neutralized are held in bags, which are separated into individual bags in use for transfusion.

Upon storage and transportation, methods and means that are applied to conventional bags can be applied as they are.